CLAIMS

1. A 2μm-family plasmid comprising a polynucleotide sequence insertion, deletion and/or substitution between the first base after the last functional codon of at least one of either a *REP2* gene or an *FLP* gene and the last base before the FRT site in an inverted repeat adjacent to said gene.

2. The 2μm-family plasmid of Claim 1 wherein, other than the polynucleotide sequence insertion, deletion and/or substitution, the *FLP* gene and/or the *REP2* gene has the sequence of a *FLP* gene and/or a *REP2* gene, respectively, derived from a naturally occurring 2μm-family plasmid.

3. The 2μm-family plasmid of Claim 1 wherein the naturally occurring 2μm-family plasmid is selected from pSR1, pSB3 or pSB4 as obtained from *Zygosaccharomyces rouxii*, pSB1 or pSB2 both as obtained from *Zygosaccharomyces bailli*, pSM1 as obtained from *Zygosaccharomyces fermentati*, pKD1 as obtained from *Kluyveromyces drosophilarum*, pPM1 as obtained from *Pichia membranaefaciens*, and the 2μm plasmid as obtained from *Saccharomyces cerevisiae*.

4. The 2μm-family plasmid of Claim 2 or 3 wherein the sequence of the inverted repeat adjacent to said *FLP* and/or *REP2* gene is derived from the sequence of the corresponding inverted repeat in the same naturally occurring 2μm-family plasmid as the sequence from which the gene is derived.

5. The 2μm-family plasmid of any one of Claims 2 to 4 wherein the naturally occurring 2μm-family plasmid is the 2μm plasmid as obtained from *Saccharomyces cerevisiae*.

6. The 2μm-family plasmid of Claim 5 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs at a position between the first base of

codon 59 of the *REP2* gene and the last base before the FRT site in the adjacent inverted repeat.

7. The 2μm-family plasmid of Claim 5 or 6 wherein, other than the polynucleotide sequence insertion, deletion and/or substitution, the sequence of the *REP2* gene and the adjacent inverted repeat is as defined by SEQ ID NO:1 or variant thereof.

8. The 2μm-family plasmid of any one of Claims 1 to 7 wherein polynucleotide sequence insertion, deletion and/or substitution occurs at a position between the first base of the inverted repeat and the last base before the FRT site.

9. The 2μm-family plasmid of any one of Claims 1 to 7 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs between the first base after the end of the *REP2* coding sequence and the last base before the FRT site, such as at the first base after the end of the *REP2* coding sequence.

10. The 2μm-family plasmid of any one of Claims 1 to 7 wherein, other than the polynucleotide sequence insertion, deletion and/or substitution, the inverted repeat that follows the *REP2* coding sequence has a sequence derived from the corresponding region of the 2μm plasmid as obtained from *Saccharomyces cerevisiae* and preferably the polynucleotide sequence insertion, deletion and/or substitution occurs at an *XcmI* site or an *FspI* site within the inverted repeat.

11. The 2μm-family plasmid of Claim 5 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs at a position between the first base of codon 344 of the *FLP* gene and the last base before the FRT site in the adjacent inverted repeat.

12. The 2μm-family plasmid of Claim 5 or 11 wherein, other than the polynucleotide sequence insertion, deletion and/or substitution, the sequence of the *FLP* coding sequence and the adjacent inverted repeat is as defined by SEQ ID NO:2 or variant thereof.

13. The 2μm-family plasmid of Claim 11 or 12 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs at a position between the first base of the inverted repeat and the last base before the FRT site.

14. The 2μm-family plasmid of Claim 13 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs at a position between the first base after the end of the *FLP* coding sequence and the last base before the FRT site.

15. The 2μm-family plasmid of Claim 14 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs at the first base after the end of the *FLP* coding sequence.

16. The 2μm-family plasmid of any one of Claims 11 to 15 wherein, other than the polynucleotide sequence insertion, deletion and/or substitution, the inverted repeat that follows the *FLP* gene has a sequence derived from the corresponding region of the 2μm plasmid as obtained from *Saccharomyces cerevisiae*, and preferably the polynucleotide sequence insertion, deletion and/or substitution occurs at an *HgaI* site or an *FspI* site within the inverted repeat.

17. The 2μm-family plasmid of any one of the preceding claims comprising polynucleotide sequence insertions, deletions and/or substitutions between the first bases after the last functional codons of both of the *REP2* gene and the *FLP* gene and the last bases before the FRT sites in the inverted repeats adjacent to each of said genes, which polynucleotide sequence insertions, deletions and/or substitutions can be the same or different.

18. The 2μm-family plasmid of any preceding claim additionally comprising a polynucleotide sequence insertion, deletion and/or substitution which is not at a position as defined in any one of the preceding claims.

19. The 2μm-family plasmid of Claim 18 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs within an untranscribed region around an ARS sequence.

20. The 2μm-family plasmid of any one of the preceding claims wherein the, or at least one, polynucleotide sequence insertion, deletion and/or substitution is a polynucleotide sequence insertion.

21. The 2μm-family plasmid of Claim 20 in which the polynucleotide sequence insertion encodes an open reading frame.

22. The 2μm-family plasmid of Claim 21 in which the open reading frame encodes a non- 2μm-family plasmid protein.

23. The 2μm-family plasmid of Claim 22 in which the non- 2μm-family plasmid protein comprises the sequence of a protein involved in protein folding, or which has chaperone activity or is involved in the unfolded protein response, albumin, a monoclonal antibody, an etoposide, a serum protein (such as a blood clotting factor), antistasin, a tick anticoagulant peptide, transferrin, lactoferrin, endostatin, angiostatin, collagens, immunoglobulins or immunoglobulin-based molecules or fragments of either (e.g. a dAb, Fab' fragments, F(ab')2, scAb, scFv or scFv fragment), a Kunitz domain protein, interferons, interleukins, IL10, IL11, IL2, interferon α species and sub-species, interferon β species and sub-species, interferon γ species and sub-species, leptin, CNTF, $CNTF_{Ax15}$, IL1-receptor antagonist, erythropoietin (EPO) and EPO mimics, thrombopoietin (TPO) and TPO mimics, prosaptide, cyanovirin-N, 5-helix, T20 peptide, T1249 peptide, HIV gp41, HIV gp120, urokinase, prourokinase, tPA, hirudin, platelet derived growth factor, parathyroid hormone, proinsulin, insulin, glucagon, glucagon-like peptides, insulin-like growth factor, calcitonin, growth hormone, transforming growth factor β, tumour necrosis factor, G-CSF, GM-CSF, M-CSF, FGF, coagulation factors in both pre and active forms, including but not limited to plasminogen, fibrinogen, thrombin, pre-thrombin, pro-thrombin, von

Willebrand's factor, α_1-antitrypsin, plasminogen activators, Factor VII, Factor VIII, Factor IX, Factor X and Factor XIII, nerve growth factor, LACI, platelet-derived endothelial cell growth factor (PD-ECGF), glucose oxidase, serum cholinesterase, aprotinin, amyloid precursor protein, inter-alpha trypsin inhibitor, antithrombin III, apo-lipoprotein species, Protein C, Protein S, or a variant or fragment of any of the above.

24. The 2μm-family plasmid of Claim 23 in which the non- 2μm-family plasmid protein comprises the sequence of albumin, a variant or fragment thereof, or a fusion protein comprising the sequence of any of these.

25. The 2μm-family plasmid of Claim 23 in which the non- 2μm-family plasmid protein comprises the sequence of transferrin, a variant or fragment thereof, or a fusion protein comprising the sequence of any of these.

26. The 2μm-family plasmid of Claim 23 in which the non- 2μm-family plasmid protein comprises the sequence of lactoferrin, a variant or fragment thereof, or a fusion protein comprising the sequence of any of these.

27. The 2μm-family plasmid of Claim 23 in which the non- 2μm-family plasmid protein comprises the sequence of Fc, a variant or fragment thereof, or a fusion protein comprising the sequence of any of these.

28. The 2μm-family plasmid of Claim 23 in which the non- 2μm-family plasmid protein comprises the sequence of a protein involved in protein folding, or which has chaperone activity or is involved in the unfolded protein response as encoded by any one of *AHA1*, *CCT2*, *CCT3*, *CCT4*, *CCT5*, *CCT6*, *CCT7*, *CCT8*, *CNS1*, *CPR3*, *CPR6*, *EPS1*, *ERO1*, *EUG1*, *FMO1*, *HCH1*, *HSP10*, *HSP12*, *HSP104*, *HSP26*, *HSP30*, *HSP42*, *HSP60*, *HSP78*, *HSP82*, *JEM1*, *MDJ1*, *MDJ2*, *MPD1*, *MPD2*, *PDI1*, *PFD1*, *ABC1*, *APJ1*, *ATP11*, *ATP12*, *BTT1*, *CDC37*, *CPR7*, *HSC82*, *KAR2*, *LHS1*, *MGE1*, *MRS11*, *NOB1*, *ECM10*, *SSA1*, *SSA2*, *SSA3*, *SSA4*,

SSC1, *SSE2*, *SIL1*, *SLS1*, *UBI4*, *ORM1*, *ORM2*, *PER1*, *PTC2*, *PSE1* and *HAC1* or a truncated intronless *HAC1*.

29. The 2μm-family plasmid of Claim 23 or 28 in which the chaperone is protein disulphide isomerase (PDI), or is a protein encoded by *ORM2*, *SSA1* or *PSE1*.

30. The 2μm-family plasmid of any one of Claims 22 to 29 in which the non- 2μm-family plasmid protein comprises a secretion leader sequence.

31. The 2μm-family plasmid of Claim 22 in which the non- 2μm-family plasmid protein comprises the sequence of a bacterial selectable marker and/or a yeast selectable marker.

32. The 2μm-family plasmid of Claim 31 in which the bacterial selectable marker is a β-lactamase gene and/or the yeast selectable marker is a *LEU2* selectable marker.

33. The 2μm-family plasmid according to any one of the preceding claims which plasmid comprises (i) a heterologous sequence encoding a non- 2μm-family plasmid protein; (ii) a heterologous sequence encoding a protein comprising the sequence of a protein involved in protein folding, a chaperone or a protein involved in the unfolded protein response, preferably protein disulphide isomerase; and (iii) a heterologous sequence encoding a protein comprising the sequence of a selectable marker; wherein at least one of the heterologous sequences occurs at a position as defined by any one of Claims 1 to 16.

34. A method of preparing a plasmid as defined by any one of the preceding claims comprising –

(a) providing a plasmid comprising the sequence of a *REP2* gene and the inverted repeat that follows the *REP2* gene, or a *FLP* gene and the inverted repeat that follows the *FLP* gene, in each case the inverted repeat comprising an FRT site;

(b) providing a polynucleotide sequence and inserting the polynucleotide sequence into the plasmid at a position as defined in any one of Claims 1 to 16; and/or

(c) deleting some or all of the nucleotide bases at the positions defined in any one of Claims 1 to 16; and/or

(d) substituting some or all of the nucleotide bases at the positions defined in any one of Claims 1 to 16 with alternative nucleotide bases.

35. A plasmid obtainable by the method of Claim 34.

36. A host cell comprising a plasmid as defined by any one of Claims 1 to 33 and 35.

37. A host cell according to Claim 36 which is a yeast cell.

38. A host cell according to Claim 36 or 37 in which the plasmid is stable as a multicopy plasmid.

39. A host cell according to Claim 38 in which the plasmid is based on pSR1, pSB3 or pSB4 and the yeast cell is *Zygosaccharomyces rouxii*, the plasmid is based on pSB1 or pSB2 and the yeast cell is *Zygosaccharomyces bailli*, the plasmid is based on pSM1 and the yeast cell is *Zygosaccharomyces fermentati*, the plasmid is based on pKD1 and the yeast cell is *Kluyveromyces drosophilarum*, the plasmid is based on pPM1 and the yeast cell is *Pichia membranaefaciens* or the plasmid is based on the 2μm plasmid and the yeast cell is *Saccharomyces cerevisiae* or *Saccharomyces carlsbergensis*.

40. A host cell according to Claim 38 or 39 in which, if the plasmid contains, or is modified to contain, a selectable marker then stability, as measured by the loss of the marker, is at least 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9% or substantially 100% after 5 generations.

41. A method of producing a protein comprising the steps of –

(a) providing a plasmid as defined by any one of Claims 1 to 33 or 35

(b) providing a suitable host cell;

(c) transforming the host cell with the plasmid; and

(d) culturing the transformed host cell in a culture medium;

(e) thereby to produce the protein.

42. A method of producing a protein comprising the steps of providing a host cell as defined by any one of Claims 36 to 40 which host cell comprises a plasmid as defined by any one of Claims 1 to 33 or 35 and culturing the host cell in a culture medium thereby to produce the protein.

43. A method according to Claim 41 or 42 further comprising the step of isolating the thus produced protein from the cultured host cell or the culture medium.

44. A method according to Claim 43 further comprising the step of purifying the thus isolated protein to a commercially acceptable level of purity.

45. A method according to Claim 44 further comprising the step of formulating the thus purified protein with a carrier or diluent, and optionally presenting the thus formulated protein in a unit form.

46. A method according to Claim 43 further comprising the step of purifying the thus isolated protein to a pharmaceutically acceptable level of purity.

47. A method according to Claim 44 further comprising the step of formulating the thus purified protein with a pharmaceutically acceptable carrier or diluent and optionally presenting the thus formulated protein in a unit dosage form.

48. A plasmid comprising, as the sole yeast selectable marker, a gene encoding a protein that is essential to the viability of a host yeast cell, in the sense that when the gene or genes encoding the essential protein are deleted or inactivated in a host yeast cell, then the host cell is inviable in culture and the deficiency cannot be complemented by additions or modifications to the culture medium.

49. The plasmid of Claim 48 wherein the gene encoding the essential protein is the sole selectable marker encoded by the plasmid.

50. The plasmid of Claim 48 or 49 wherein the essential protein is a chaperone.

51. The plasmid of 51, wherein the chaperone is protein disulphide isomerase or Pselp.

52. The plasmid of any one of Claims 48 to 51 further comprising a gene encoding a non- 2μm-family plasmid protein, such as a protein defined by any one of Claims 23 to 27.

53. The plasmid of any one of Claims 48 to 52 which is a 2μm-family plasmid.

54. The plasmid of Claim 53 which is a plasmid as defined by any one of Claims 1 to 33.

55. A host cell comprising a plasmid, the plasmid comprising a gene that encodes protein that is essential to the viability of the host cell and wherein, in the absence of the plasmid, the host cell is unable to produce the essential protein and cannot be made viable by additions or modifications to the culture medium.

56. A host cell according to Claim 55 in which the plasmid is a plasmid according to any one of Claims 1 to 33 or 48 to 54.

57. A host cell according to Claim 55 or 56 wherein, in the absence of the plasmid, the host cell is inviable.

58. The host cell of any one of Claims 55 to 57 wherein the host cell further comprises a recombinant gene encoding a desired protein, such as a protein defined by any one of Claims 23 to 27.

59. The host cell of Claim 58 wherein the desired protein is a non- 2μm-family plasmid protein defined by any one of Claims 23 to 27.

60. The host cell of Claim 59 wherein the essential protein is a chaperone, such as protein disulphide isomerase or PSE1.

61. The host cell of Claim 59 or 60 wherein the recombinant gene is located on the same plasmid as the gene encoding the essential protein.

62. A method for producing a desired recombinant protein comprising the steps of: providing a host cell as defined by any one of Claims 59.to 61; culturing the host cell in a culture medium under conditions that allow the expression of the essential protein and the desired protein; and optionally isolating the thus expressed desired protein from the cultured host cell or the culture medium; and optionally purifying the thus isolated desired protein to a commercially acceptable level of purity; and further optionally, lyophilising the thus purified protein or formulating the purified desired protein with a carrier or diluent (such as a pharmaceutically acceptable carrier or diluent); and optionally presenting the thus formulated desired protein in a unit dosage form.

63. The method of Claim 62 wherein the step of culturing the host cell involves culturing the host cell in a non-selective media, such as a complex or rich media.